Insect background sodium channel as a new target for scorpion alpha toxin†

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Abstract: The effects of an anti-insect scorpion alpha toxin, LqhαIT, isolated from the venom of the Israeli scorpion *Leiurus quinquestriatus hebraeus* was studied on isolated adult dorsal unpaired median (DUM) neurones isolated from the cockroach *Periplaneta americana* terminal abdominal ganglion. Using the cell-attached patch-clamp configuration, a new type of sodium channel, called background sodium channel (bNa), was recently characterized. At −50 mV, the channel activity was observed as unclustered brief single openings. For hyperpolarized steady-state holding potential (−100 mV) the patches contained large unitary current steps, appearing generally in bursts. The open probability (Po) calculated at −50 mV was low (0.008 (±0.004), $n = 5$) and displayed a typical bell-shaped voltage dependence. LqhαIT (10^{-8} M) altered the bNa activity in a time-dependent manner. At −50 mV the channel activity appeared in bursts. Po calculated at −50 mV was about 20 times greater than Po calculated in controls and also showed bell-shaped voltage dependence. At 10^{-7} M, LqhαIT induced longer silent periods interrupted by bursts of increased channel activity. Whole-cell recordings revealed that 10^{-7} M LqhαIT transformed regular beating DUM neurone pacemaker activity into a rhythmic bursting. In this paper we demonstrate, for the first time, that bNa is a new target for anti-insect scorpion toxin.

Keywords: background Na channels; scorpion alpha toxin; *Periplaneta americana*

1 INTRODUCTION

Rapid paralysis or knock-down following application of toxins or insecticides is the result of disruption of normal functioning of voltage-dependent sodium channels in insects.[1,2] It is thought that sodium channels are the primary site of action because of their highly conserved function.

Three groups of peptide neurotoxins derived from scorpion venoms exhibit a preferential higher activity on insects than on mammals.[3–5] Among them the scorpion alpha-toxin LqhαIT, from *Leiurus quinquestriatus hebraeus*, like other classical alpha toxins, slows down inactivation of the voltage-dependent sodium current in the cockroach giant axon.[6] Recently, another type of sodium channel activity has been characterized in adult neurosecretory dorsal unpaired median (DUM) neurones, isolated from the terminal abdominal ganglion (TAG) of the cockroach *Periplaneta americana* L.[7] These channels, named background sodium channels, show some electrophysiological characteristics different from those described for the classical voltage-dependent sodium channels expressed in the same preparation.[8] It has been suggested that the DUM neurone background sodium channels could be involved in driving the membrane potential to threshold for action potential generation.[9]

In this paper we report an investigation with the action of LqhαIT on the background sodium channels of cockroach DUM neurones. In particular, we were interested in its specific mode of action on these channels as a potential new target site for insecticides and its effect on beating pacemaker activity.

2 EXPERIMENTAL METHODS

Experiments were performed on DUM neurone cell bodies isolated from the midline of the TAG of the

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nerve cord of adult male cockroaches, *P americana*, taken from our stock colonies maintained at 29 °C with a photoperiod of 12 h light –12 h dark. Isolation of adult DUM neurone cell bodies was performed under sterile conditions using enzymatic digestion and mechanical dissociation of the median parts of the TAG from the nerve cord of the cockroach as previously described.[9,10]

2.1 Single channel recording

Single background sodium channel activity was recorded using the cell-attached configuration of the patch-clamp technique.[11] Patch pipettes were pulled from borosilicate glass capillary tubes (Clark Electromedical Instruments, Reading, UK) and heat-polished to 2–5 MΩ when filled with solution (see Section 2.3). Patch pipettes were coated with Sylgard (Dow Corning Corporation, Midland, USA) to reduce stray capacitance. The liquid junction potential between bath and internal pipette solution was always corrected before the formation of a gigaohm seal (>30 GΩ). Current was recorded using an Axopatch 200A (Axon Instruments, Foster City, CA) amplifier, filtered at 2 kHz. These signals were stored on-line on the hard disk of an IBM Pentium 100 computer connected to a 125-kHz labmaster DMA data acquisition system (TL-1-125 interface, Axon Instruments). The pClamp package (version 6.03, Axon Instruments) was used for data acquisition and analysis (sampling frequency 12.50 kHz). Transitions from closed to open states were determined by a 50% threshold detection method.

2.2 Whole-cell recording

The whole cell patch-clamp technique was used to record spontaneous action potentials and membrane potential. Patch electrodes had resistances ranging from 1 to 3 MΩ when filled with the pipette solution (Section 2.3). Action potentials were displayed on a digital oscilloscope (310, Nicolet Instrument Corporation, Madison, WI, USA) and stored on a Digital Tape Recorder 1202 (Biologic, Claix, France) for subsequent off-line analysis.

2.3 Solutions

For single channel recording, the pipette solution contained (mM): sodium chloride, 200; calcium chloride, 0.5; tetraethylammonium (TEA) chloride, 10; Hepes, 10; pH was adjusted to 7.4 with sodium hydroxide. During formation of the seal, the DUM neurone cell bodies were initially superfused with an external saline containing (mM): sodium chloride, 100; TEA-Cl, 100; potassium chloride, 3.1; calcium chloride, 2; magnesium chloride, 7; cadmium chloride, 1; 4-aminopyridine, 4; Hepes, 10; pH was adjusted to 7.4 with TEAOH. Once the seal was established, a high-potassium external solution was used to lower the membrane potential to close to 0 mV. Thus the command potentials applied during cell-attached recordings were easily converted to patch potentials. This solution contained (mM): potassium aspartate, 170; sodium chloride, 30; magnesium chloride, 4; EGTA, 5; Hepes, 20; pH was adjusted to 7.4 with potassium hydroxide. For whole-cell recording, the bathing solution contained (mM): sodium chloride, 200; potassium chloride, 3.1; calcium chloride, 5; magnesium chloride, 4; Hepes, 10; pH was adjusted to 7.4 with sodium hydroxide. The patch electrode was filled with a solution of the following composition (mM): potassium chloride, 170; sodium chloride, 15; ATP-Mg, 3; EGTA, 10; calcium chloride, 0.5; Hepes, 10; pH was adjusted to 7.4 with potassium hydroxide. All compounds were purchased from Sigma Chemicals (L'isle d'Abeau Chesnes, France). The scorpion alpha toxin LqhαIT isolated from the venom of the Israeli scorpion *L quinquestriatus hebraeus*[3] was dissolved in the saline at the final concentration just before use. Experiments were carried out at room temperature (21 °C). Data, when quantified, were expressed as mean (±SEM).

3 RESULTS

As illustrated in Fig 1A, DUM neurone background sodium channel activity recorded in control preparations at a steady-state holding potential of −50 mV, occurred as unclustered, brief single openings with a mean amplitude of −3.93 (±0.21) pA (n=5). The background channel, whose main biophysical properties have already been characterized,[7] has a very low open probability (Po, 0.008 (±0.004; n=5) which displays a bell-shaped voltage dependence (Fig 2A).

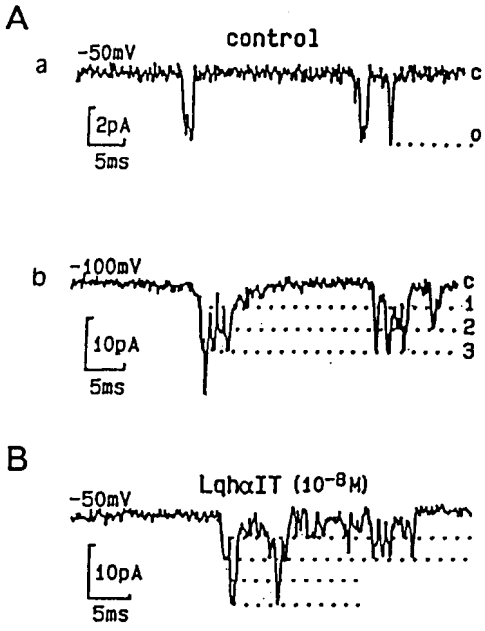

Figure 1. A(a, b): Background sodium channel currents (recorded under the cell-attached patch-clamp configuration) of adult DUM cell body recorded at two steady-state holding potentials indicated above each trace. The sample frequency is 12.5 kHz. **C**: closed state, **O**: 1, 2, 3: open current levels. B: Example trace of inward sodium current recorded at a holding potential of −50 mV, after 6 min of toxin application with a patch pipette containing 10^{-8} M LqhαIT.

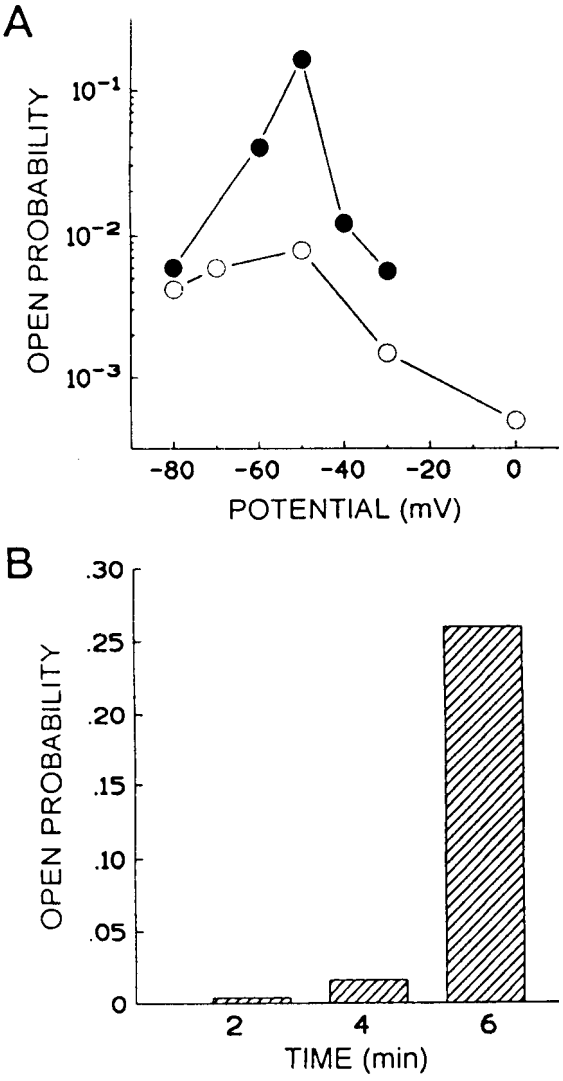

Figure 2. A: Graph illustrating the voltage dependence of the open probability (Po) of background sodium channel calculated in control conditions (○, $n=5$) and after 10^{-8}M LqhαIT treatment (●, $n=4$), plotted as a function of the steady-state holding potentials. B: Histogram obtained from a typical experiment illustrating the time-dependent effect of 10^{-8}M LqhαIT on the Po calculated at a steady-state holding potential of −50mV, 2, 4 and 6min after toxin application.

Po is maximum at −50mV (corresponding to the resting membrane potential of DUM neurones)[9] and decreases for lower and higher steady-state holding potentials. Steady-state hyperpolarization to −100mV, for instance, transforms channel activity into large unitary current steps, due to superimposed channel activity (Fig 1Ab). In this case, the channel activity appears in bursts with rapid flickering between different open and closed states.

3.1 Effect of LqhαIT on background sodium channel

Figure 1B illustrates the effect of LqhαIT on DUM neurone background sodium current recorded at −50mV. LqhαIT (10^{-8}M) applied on the external side of the membrane patch, induced a time-dependent effect (Fig 2B). During the first two minutes, LqhαIT did not modify the behaviour of the background sodium channel. After four minutes, channel openings occured more frequently (Fig 2B) and after five to six minutes of 10^{-8}M toxin application, the background sodium channel activity occured in irregular bursts, which were absent at −50mV in control preparations. This bursting activity was separated by short silent periods during which isolated brief single events were detected (Fig 1B). Within a burst, the channel activity fluctuated between the closed state and up to five open current levels. The effect of LqhαIT on background sodium channel has been examined further to determine whether the toxin altered the Po or the voltage dependence of Po. As illustrated in Fig 2A, 10^{-8}M LqhαIT increased the channel open probability (Po). At −50mV, for example, Po was about 20 times greater (from 0.008 to 0.16) than the Po determined in the control. It is interesting to note that the bell-shaped voltage dependence of Po was not altered during LqhαIT treatment and declined for lower and higher steady-state holding potential. The maximum Po remained at −50mV (mean value of Po=0.17 (±0.06), $n=4$). It should be noted that 10^{-8}M LqhαIT did not modify the distribution of channel open time, (0.22 (±0.02) ms; $n=4$) calculated at −50mV, compared with the value found in control conditions.[7]

Higher concentration of LqhαIT (10^{-7}M) dramatically altered the background channel activity. The bursts of increased channel activity were more condensed and separated by longer (>3s) silent periods than with a LqhαIT concentration 10 times lower (Fig 3A compared to Fig 1B). This was confirmed by the scatter plot of current amplitude *versus* time (Fig 3B) indicating a heterogenous distribution for the duration of the recording. In parallel, the increased Po was not homogenous and constant during the recording but appeared in bursts of high Po (Fig 3C). This demonstrates the close relationship between bursts of increased channel activity and the Po measured at −50mV.

3.2 Effect of LqhαIT on beating pacemaker activity

We have also studied the physiological consequences of the LqhαIT-modified background sodium channels on the beating pacemaker activity of DUM neurones. Under control conditions, TAG DUM neurones are known to fire repetitive spontaneous action potentials at regular intervals.[9] Figure 4A illustrates typical spontaneous action potentials (6–10Hz in frequency) recorded from whole cells. Bath application of 10^{-7}M LqhαIT induced a slight depolarization (about 5mV) and transformed the regular firing into an activity pattern consisting of bursts (containing about six spikes), separated by long hyperpolarization of the membrane (>5s), resulting in the disappearance of spontaneous electrical activity (Fig 4B). Hyperpolarization observed between the bursts of action potentials seemed to be correlated with the silent periods

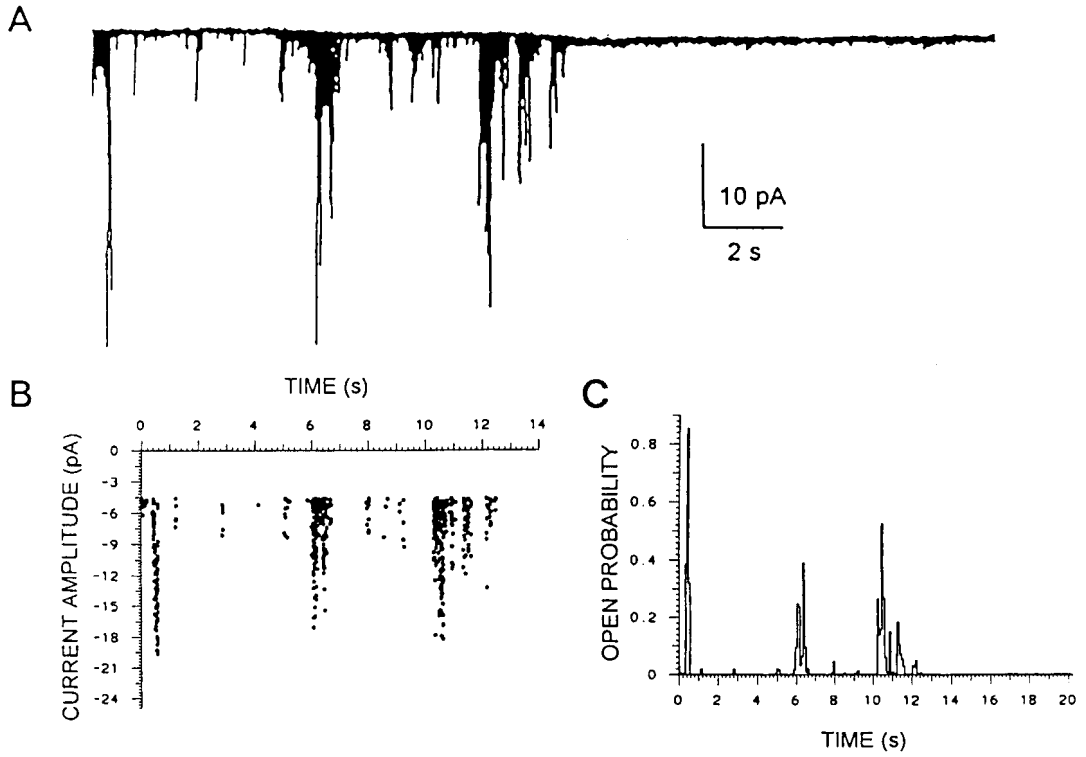

Figure 3. Effect of 10^{-7} M LqhαIT on DUM neurone background sodium channel activity. A: Selected current trace recorded at a steady-state holding potential of −50 mV. B and C are the corresponding scatter plots of background current amplitude *versus* time and the channel open probability (Po) plotted against time, respectively. Vertical bars in the last case represent the channel Po.

separating two bursts of increased background channel activity (Fig 3A). LqhαIT used at this concentration did not affect the different phases of action potentials as was observed, for instance, with scorpion alpha toxin in cockroach axonal membrane.[6] This indicated that the classical voltage-dependent sodium current was not affected by low concentration of scorpion alpha toxin. However, application of 10^{-6} M LqhαIT was able to slow down inactivation of the voltage-dependent sodium current in TAG DUM neurones.[6]

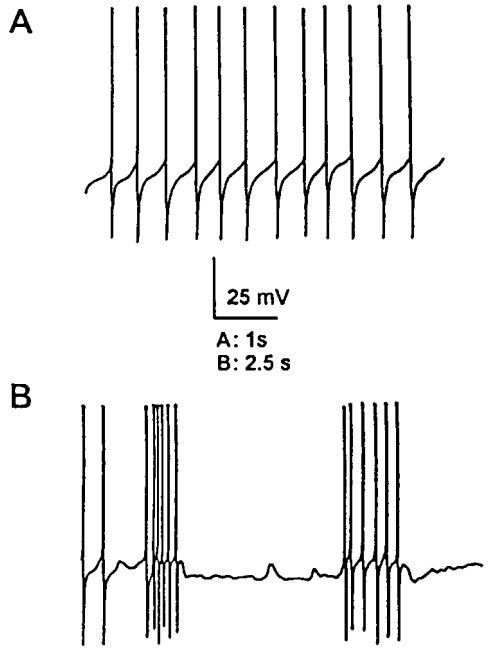

Figure 4. Spontaneous electrical activity recorded with the patch-clamp technique (whole-cell configuration) in an isolated adult DUM neurone cell body. A: Beating pacemaker activity recorded under control conditions. B: Spontaneous bursting activity recorded after bath application of 10^{-7} M LqhαIT.

4 DISCUSSION

It is well established that scorpion and sea anemone alpha toxins specifically modify voltage-dependent sodium channel gating by interacting with the inactivation mechanism. The alpha toxins are known to slow down the global sodium current inactivation on vertebrate as well as invertebrate neuronal voltage-dependent sodium channels.[12–14] At a single channel level, they prolong the mean open time, induce repetitive openings without affecting the channel ionic selectivity[15,16] and recently it has been shown that, upon a depolarization, sea anemone toxin II-modified sodium channels could rapidly enter into a high-conductance state and more slowly into a low-conductance state.[17]

In cockroach TAG DUM neurones, two types of sodium current have been described, the voltage-dependent sodium current responsible for the depolarizing phase of action potential[8] and the background sodium current essential to maintain the beating pacemaker activity of these neurones.[7,9] In this paper, we demonstrate for the first time the existence of a new type of target for the well-known

scorpion alpha toxin LqhαIT. Very low concentrations of LqhαIT preferentially affected the background sodium channels without any effect on the voltage-dependent sodium channels. It was necessary to use a concentration of LqhαIT 100 times higher to observe an effect on the voltage-dependent sodium current on this preparation. The effect of LqhαIT is unusual. This scorpion alpha toxin modified homogenous distribution of the channel activity which characterizes the behaviour of the background channels.[7] LqhαIT causes the development of a bursting channel activity in the range of normal resting membrane potential (ie −50 mV) by increasing the number of open current levels separated by several closures to different intermediate levels and/or to the zero current level. In contrast, at −50 mV, bursting activity was always absent in the control. However, at very negative steady-state holding potentials (eg −100 mV), different current levels were observed, such as those seen under scorpion alpha toxin treatment. This similarity leads us to suggest the existence of a complex mechanism of background sodium channels that involves simultaneous openings of several conducting units forming the channel, as has already been proposed for other background channels,[18,19] most of these units being 'inactivated' (ie, not functional) at −50 mV. Hyperpolarization or LqhαIT treatment at −50 mV could enhance the opening of most of these units, inducing the bursting phenomenon.

Although the classical voltage-dependent sodium channels play a fundamental role in the generation and the conduction of electrical activity, we report in this study that background sodium channels are not only involved in driving the membrane potential to threshold for action potential generation[9] but can play an additional physiological role in determining the firing pattern of pacemaker neurosecretory cells. LqhαIT is capable of transforming the regular firing pattern of TAG DUM neurones into a bursting electrical activity. This fundamental alteration of the somatic intrinsic electrical activity has already been observed in a variety of invertebrate, as well as vertebrate, neurones, and different mechanisms of such alteration have been proposed.[20–22] In our study, the transition from beating activity to rhythmic bursting seems to be due to an alteration of the background sodium channel behaviour. Correlation between the long silent periods separating two bursts of action potentials during which the neurone is often hyperpolarized and the silent periods observed between two bursts of increased channel activity is obvious. It is clear that LqhαIT is capable of inducing the switch between these two intrinsic electrical properties, probably being conditional upon changes in background channel behaviour (ie transition between homogenous distribution of single events into bursting activity of the background channels). Transformation from beating activity to rhythmic bursting in a given DUM neurone could strongly influence the neurosecretory function (eg release of octopamine, known to be involved in the modulation of different vital functions in insects).[23] Although the background sodium channels have not yet been characterized in many neuronal preparations, they may exist in other types of insect neurosecretory cells. In this case, their functional alteration may profoundly affect poisoned insects. Consequently, this high scorpion alpha toxin sensitivity makes the background sodium channels suitable candidates as primary targets for other toxins or insecticides. From our results emerges an interesting question: can insect selective toxins be used as biological control agents? The pyrethrins are well-known examples of defensive plant compounds that are toxic to insects. In the last three decades, pyrethroids have been successfully developed for controlling insect pests,[1] but the rapidly developing resistance to conventional insecticides makes it necessary to discover new targets for insecticides. Scorpion toxins and pyrethroids bind to insect voltage-dependent sodium channels at different binding sites.[24] Some cases of resistance to pyrethroids have been demonstrated to be due to point mutations in a locus encoding a voltage-gated sodium channel.[25] Because of the results reported here, it would be interesting to perform further comparative studies between native and mutant voltage-gated, as well as background, sodium channel interactions with pyrethroids and scorpion toxins. This may contribute to overcoming pyrethroid resistance. In parallel the development of new insect control methods is necessary. As an example, the baculovirus, which is an insect pathogen, has been used as an expression vector of anti-insect toxins. It was demonstrated that baculovirus pathogenicity could be enhanced by 30–40% by the expression of such toxins.[26,27] Furthermore, a recent study reported that baculoviruses expressing depressant and excitatory scorpion anti-insect selective toxins possessed high insecticidal activity.[28]

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